Evaluation of Oxidative Stress and Using a Novel Automated Method For Measurement of Total Antioxidant Status in Preeclampsia

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OBJECTIVE: The purpose of this study was to evaluate total antioxidant status of preeclampsia with using a more recently developed automated measurement method and measurement of malondialdehyde (MDA) levels as an oxidative stress marker in women with preeclampsia
STUDY DESIGN: We performed a case-control study consisting of randomly selected 50 healthy pregnant women and 50 patients diagnosed as preeclampsia. We measured the total plasma antioxidant capacity for all patients and oxidative stress was evaluated with measurement of red blood cell malondialdehyde (MDA) levels.
RESULTS: The serum total antioxidant response (TAR) levels were lower (1.29±0.33 TAR mmol Trolox eq/L) and the red blood cell malondialdehyde levels of (MDA) were higher (7.1±0.4 nmol MDA/l) before delivery ($P<0.001$) and these levels were not different after delivery (six weeks later) in preeclampsia than control group ($P>0.05$).
The serum total antioxidant response (TAR) levels significantly increased (1.55±0.49 mmol Trolox eq/L) and the malondialdehyde levels significantly decreased (2.9±0,6 nmol MDA/l) in preeclamptic women after delivery when compared with the pre delivery levels
CONCLUSIONS: Our findings suggest that preeclampsia is related with enhanced lipid peroxidation and decreased total antioxidant response before delivery and these parameters may be related at least partly to the pathogenesis of preeclampsia. Decreased lipid peroxidation and increased total antioxidant response after delivery may suggest recovery of preeclampsia and using a simple, rapid and reliable automated method facilitates to evaluate and follow up of the levels of oxidative stress in preeclampsia and this novel method may be used as a routine test in clinical practice while managing preeclampsia
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Key Words: Preeclampsia, Oxidative stress, Total antioxidant response, Malondialdehyde

Preeclampsia is characterized by pregnancy-induced hypertension and proteinuria, which is associated with maternal and fetal morbidity and mortality. The pathophysiology of preeclampsia is known to be associated with endothelial cell dysfunction.[1]

Oxidative stress been increasingly claimed as a major contributor to endothelial dysfunction in preeclampsia (PE).[2] Oxidative stress, resulting from the imbalance between increased reactive oxygen species (ROS) formation and defects in antioxidant defense mechanisms.[3,4] ROS (e.g. hydrogen peroxide, superoxide anion and hydroxyl radical) can be generated by living cells during many metabolic and physiological processes and these free radicals cause lipid peroxidation and have other deleterious effects on cell structure and antioxidant systems which plays a role to protect them against the harmful oxidative reactions that occur as a result of reactive oxygen species production. Oxidant/antioxidant balance shifts towards the oxidative state in preeclampsia.[5]

Lipid peroxidation, a central feature of oxidant stress, has been shown an increase in circulating lipid peroxides and decrease in antioxidant patterns which contributes to endothelial cell dysfunction in preeclampsia.[6,7] Circulating levels of malondialdehyde (MDA), one of the end products of lipid peroxidation, has been found to be elevated in preeclampsia.[8] It is important to evaluate the overall effectiveness of antioxidant defense systems in limiting peroxidative damage but the data about total responses of the serum antioxidant systems in pre-eclampsia is limited.[9]

Total antioxidant response has been measured in different studies and data about concerning this topic is conflicting. Kharb found significantly higher total antioxidant response in pre-eclampsia[10] while Shaarawy et al. found significantly lower total antioxidant response in preeclampsia.[9] Total antioxidant response can be measured different techniques such as colorimetric, fluorescence, chemiluminescence methods but these methods are sophisticated, limited and are not available in every clinical biochemistry laboratories.[11-14] Consequently there is not any accepted precise reference method for measurement of total antioxidant response.[5]

In this study, we aimed to measure the levels of individual antioxidant components, and the total antioxidant response (TAR) values in serum samples from pregnant women before delivery and six weeks after delivery to evaluate their antioxi-

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dant status using a novel automated method.[15] We also measured red blood cell plasma MDA levels as an indicator of lipid peroxidation before delivery and six weeks after delivery.

We then evaluated the possible roles of those parameters in preeclampsia, and examined the relationship between lipid peroxidation and total antioxidant response (TAR) levels in the pathogenesis and management of preeclampsia.

Materials and methods

This case-control study was carried out on 50 preeclamptic and 50 healthy normotensive pregnant women attended at the Obstetric Division of the University Hospital in Diyarbakır, Turkey. This study was performed with the approval of the Ethics Committee and all subjects were recruited in a voluntary manner, giving their written informed consent. The healthy normotensive pregnancy was diagnosed on the basis of clinical, biochemical, and ultrasound findings and. None of the patients had preexisting hypertensive disorders or any renal, hepatic, or hematologic diseases and had received no medication or vitamin supplementation and none of them were smokers.

Blood samples were obtained before any medication was taken and before the onset of labor and six weeks after delivery. Samples were withdrawn from a cubital vein into the vacutainer tubes for total antioxidant status and for lipid peroxidation into the vacutainer tubes containing ethylenediamenetetraacetic acid (EDTA). The serum was then separated from the cells by centrifugation at 3500 X g for 5 min for the total antioxidant status and tubes containing EDTA, lipid peroxidation, were centrifuged at 1500 × g for 5 min at 4°C and erythrocytes were washed three times with NaCl 0.9%. Serum and washed erythrocytes were stored at -80°C until analysis.

Measurement of the total antioxidant status of serum

The total antioxidant status of the serum was measured using a novel automated colorimetric measurement method for the total antioxidant response (TAR) developed by Erel.[15] In this method the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction, and reacts with the colourless substrate O-dianisidine to produce the dianisdyl radical, which is yellow-brown in colour. Upon the addition of a serum sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the serum, preventing the colour change and thereby providing an effective measure of the total antioxidant capacity of the serum. The assay results are expressed as mmol Trolox eq./L, and the precision of this assay is excellent, being lower than 3%.[16]

Measurement of the lipid peroxidation of red blood cell

Red blood cells of lipid peroxidation were measured as described by Stocks and Dormandy.[17] Hydrogen-peroxide induced lipid peroxidation was determined after incubation with H2O2 for 2 h at 37 °C. The final incubation mixture contained 5 mmol/L H2O2, 1.65 mmol/L NaN3 and erythrocyte suspension in phosphate-buffered saline (14 mg hemoglobin/ml of incubation mixture). Lipid peroxidation was assayed by measurement of malondialdehyde (MDA) production using 1,1,3,3-tetraethoxypropane as standard. Results were expressed as nmol MDA/g hemoglobin.

Statistical analysis

Student's t-test was performed for paired and independent means using SPSS for Windows Release 11.5 (SPSS Inc.) and $P \leq 0.05$ was considered statistically significant.

Results

The demographic and clinical data of the subjects are shown in Table 1. As expected, the women affected by preeclampsia demonstrated significantly higher systolic ($P<0.001$) and diastolic blood pressures ($P<0.001$) than women with normal pregnancies. And, the women with preeclampsia had a significantly higher mean body weight ($P<0.001$) and gave birth to babies with significantly lower birth weights ($P<0.001$). The control women did not differ from those in the preeclampsia groups regarding maternal age, gestational age, gravidity, parity ($p> 0.05$).

Table 1. Demographic and clinical characteristics of patients with preeclamptic pregnants and healthy pregnant women.

	Preeclamptic pregnancy (n=50)	Normal pregnancy (n=50)	P
Maternal age (years)	28.4±5.7	29.1±7.7	NS
Gestational age (weeks)	34.4±1.7	37.2±1.4	NS
Systolic BP (mmHg)	165±13.9	118±9.7	<0.001
Diastolic BP (mmHg)	96.3±5.2	72.8±4.7	<0.001
Gravida	2.2±0.6	2.7±0.4	NS
Parity	1.0±0.5	1.0±0.6	NS
Maternal weight (kg)	82.5±7.6	73.3±4.2	<0.001
Birth weight (g)	2557.5±162.6	3012.5±219.4	<0.001

Results are mean±S.D.; NS*, not significant according to Student's t-test.

As seen in Table 2 the serum total antioxidant response (TAR) levels of the preeclamptic patients before delivery were found to be significantly lower than those of healthy pregnant women ($p<0.001$). On the contrary, red blood cell MDA levels before delivery were significantly higher in the preeclamptic women than healthy pregnant women ($p<0.001$).

As seen in Table 3, the serum total antioxidant response (TAR) and red blood cell MDA levels of the preeclamptic patients after delivery (six weeks later) did not differ from those in the control women ($p>0.05$).

Table 2. TAR (mmol Trolox eq/L) and Malondialdehyde (nmol MDA/l) parameters of preeclamptic and healthy pregnant women before delivery.

	Preeclamptic pregnancy before delivery (n=50)	Normal pregnancy before delivery (n=50)	P
TAR (mmol Trolox eq/L)	1.29±0.33	1.74± 0.49	<0.001
Malondialdehyde (nmol MDA/l)	7.1±0.4	3.2±0.5	<0.001

Results are mean±S.D.; NS*, not significant according to Student's t-test; TAR, total antioxidant response.

Table 3. TAR (mmol Trolox eq/L) and Malondialdehyde (nmol MDA/l) parameters of preeclamptic and healthy pregnant women after delivery (six weeks later).

	Preeclamptic pregnancy after delivery (n=50)	Normal pregnancy after delivery (n=50)	P
TAR (mmol Trolox eq/L)	1.55±0.49	1.69±0.48	>0.05
Malondialdehyde (nmol MDA/l)	2.9±0.6	3.0±0.5	>0.05

Results are mean±S.D.; NS*, not significant according to Student's t-test; TAR, total antioxidant response.

Serum total antioxidant response (TAR) levels change in the preeclamptic and control groups in prepartum and after six weeks later postpartum period. The serum total antioxidant response (TAR) levels of the preeclamptic women significantly increased after delivery (1.55±0.49 mmol Trolox eq/L) when compared with the pre delivery levels (1.29±0.33 mmol Trolox eq/L) ($p<0.001$). In contrast, Serum total antioxidant response (TAR) levels were not changed statistically in control groups before (1.74± 0.49 mmol Trolox eq/L) and after delivery (1.69± 0.48 mmol Trolox eq/L) ($p>0.05$) (Figure 1).

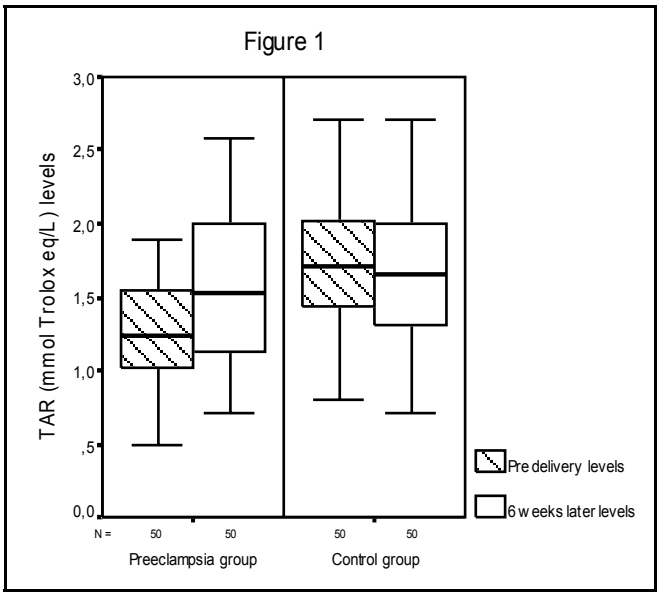

Figure 1. Serum total antioxidant response (TAR) levels change in the preeclamptic and control groups in prepartum and after six weeks later postpartum period.

MDA levels change in the preeclamptic and control groups before and after delivery (six weeks later). The MDA levels of the preeclamptic women significantly decreased after delivery (7.1±0.4 nmol MDA/l) when compared with the pre delivery levels (2.9±0,6 nmol MDA/l) ($p<0.001$). In contrast , The MDA levels were not changed statistically in control groups before (3.0± 0.5 nmol MDA/l) and after delivery (3.2± 0.5 nmol MDA/l) ($p>0.05$) (Figure 2).

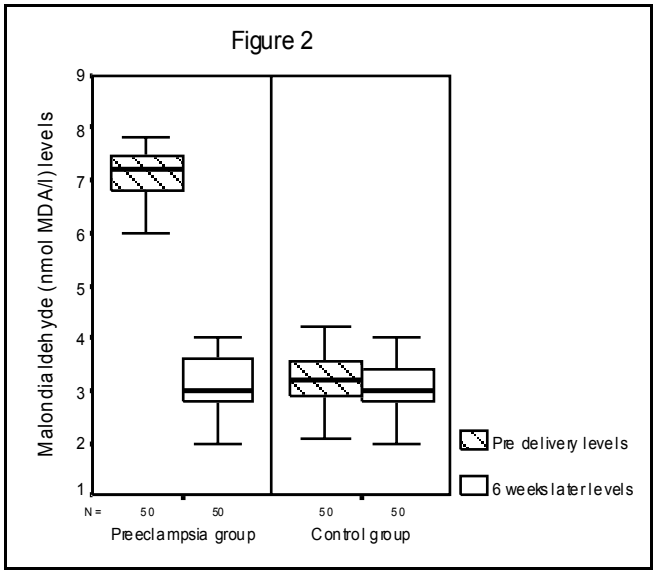

Figure 2. Malondialdehyde (MDA) levels change in the preeclamptic and control groups in prepartum and after six weeks later postpartum period.

Discussion

Hypertensive disorders of pregnancy complicate about 7-10% of all pregnancies[18] Preeclampsia is a hypertensive disorder of pregnancy, characterized by vasospasm, proteinuria, edema and is the leading cause of maternal, fetal and neonatal mortality and morbidity.[19]

The pathophysiology of the disease remains poorly understood. According to the most recent hypothesis, preeclampsia is a generalized inflammatory state that provokes endothelial hyperstimulation which leads to severe endothelial dysfunction, and results in disseminated microangiopathic disease with vasospasm and hypercoagulation.[19,20]

Free radical generation contributes to endothelial dysfunction in preeclampsia which is supported by studies

showing an increase in circulating lipid peroxides and a decrease in antioxidant patterns in preeclamptic patients.[6,7,21] Preeclampsia is associated with oxidative stress, defined as an imbalance between prooxidant and antioxidant factors in favor of prooxidants.[22,23]

Measurements of antioxidants status of preeclamptic patients can be made seperately in the laboratory, but these measurements are time-consuming, labour intensive and costly. Since the effects of the antioxidant components in plasma are additive, measurement of the total antioxidant response accurately reflects the redox status of the plasma.[3] Thus, instead of measurement of individual antioxidant components of plasma as single tests, the TAR may be more useful and practical to evaluate the antioxidant status of serum. The most widely used methods for measurement of TAR are either colorimetric, fluorescence-based, or chemiluminescence[11-13] are not appropriate for routine usage. However, until now there is no accepted, "gold standard" reference method but a novel automated method devoloped by Erel[15] has several major advantages over the other techniques. It is simple and inexpensive, and can easily be fully automated. It is also reliable and sensitive, and is not subject to interference by commonly occurring serum components such as bilirubin, serum lipids, and anticoagulants such as heparin or oxalate.[15] Accurate measurements of the total serum antioxidant response can be obtained in as little as 10 min, making this assay suitable for the clinical biochemistry laboratory.

In our study, we have used this novel measurement method to evaluate the total antioxidant capacity of preeclamptic patients before and after delivery, and compared with those of normal, healthy pregnant women. The serum total antioxidant response (TAR) levels of the preeclamptic patients before delivery were found to be significantly lower (1.29±0.33 mmol Trolox eq/L) than those of healthy pregnant women (1.74± 0.49 mmol Trolox eq/L) ($p<0.001$). On the contrary, the serum total antioxidant response (TAR) levels of the preeclamptic patients after delivery (six weeks later) did not differ (1.55±0.49 mmol Trolox eq/L) from those in the control women (1.69± 0.48 mmol Trolox eq/L) ($p>0.05$). Moreover, the serum total antioxidant response (TAR) levels of the preeclamptic women significantly increased after delivery when compared with the pre delivery levels ($p<0.001$).

MDA, a terminal compound of lipid peroxidation, is used widely as an index of oxidative status.[8] A number of reports indicate that lipid peroxidation products such as MDA are elevated in women with preeclampsia relative to normal pregnancy[7] Yoshio Yoneyama et al[8] measured plasma MDA level of preeclampsia which was significantly higher than normal pregnancy

In our study, we measured the red blood cell MDA levels of preeclamptic patients before and after delivery as an oxidative stress marker and compared with those of normal, healthy pregnant women. The red blood cell MDA levels of the preeclamptic patients before delivery were found to be significantly higher (7.1±0.4 nmol MDA/l) than those of healthy pregnant women (3.2±0.5 nmol MDA/l) ($p<0.001$). On the contrary, the red blood cell MDA levels of the preeclamptic patients after delivery (six weeks later) did not differ (2.9±0.6 nmol MDA/l) from those in the control women (3.0±0.5 nmol MDA/l) ($p>0.05$). However, the red blood cells MDA levels of the preeclamptic patients significantly decreased after delivery when compared with the pre delivery levels ($p<0.001$).

In our study, we have used a novel automated method to measure total antioxidant response (TAR) against potent free radical reactions to evaluate the degree of oxidative stress in preeclamptic patients before and after delivery, and compared with healthy pregnant women. It is a useful and rapid method for evaluation of the total antioxidant response (TAR) status and the determination of an appropriate treatment management plan of preeclamptic patients. We observed an increase in total antioxidant response (TAR) and decrease in red blood cell MDA levels of preeclamptic patients after delivery (six weeks later) which may suggest recovery of preeclampsia.

In our study, significantly elevated MDA levels of red blood cells of preeclamptic pregnancies may contribute to pathophysiology and pathogenesis at preeclampsia.

In conclusion, measurement of serum TAR levels as a routine and rapid test may be useful during the management and postpartum period of preeclampsia.

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